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Review article

Review: High-throughput phenotyping to enhance the use of crop genetic resources☆

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ABSTRACT

Improved genetic, genomic and statistical technologies have increased the capacity to enrich breeding populations for key alleles underpinning adaptation and continued genetic gain. In turn, directed genomic selection together with increased heritability will reduce genetic variance to narrow the genetic base in many crop breeding programs. Diverse genetic resources (GR), including wild and weedy relatives, landraces and reconstituted synthetics, have potential to contribute novel alleles for key traits. Targeted trait identification may also identify genetic diversity in addressing new challenges including the need for modified root architecture, greater nutrient-use efficiency, and adaptation to warmer air and soil temperatures forecast with climate change. Yet while core collections and other GR sources have historically been invaluable for major gene control of disease and subsoil constraints, the mining of genetically (and phenotypically) complex traits in GR remains a significant challenge owing to reduced fertility, limited seed quantities and poor adaptation through linkage drag with undesirable alleles. High-throughput field phenomics (HTFP) offers the opportunity to capture phenotypically complex variation underpinning adaptation in traditional phenotypic selection or statistics-based breeding programs. Targeted HTFP will permit the reliable phenotyping of greater numbers of GR-derived breeding lines using smaller plot sizes and at earlier stages of population development to reduce the duration of breeding cycles and the loss of potentially important alleles with linkage drag. Two key opportunities are highlighted for use of HTFP in selection among GR-derived wheat breeding lines for greater biomass and stomatal conductance through canopy temperature.

1. Introduction

Farmers rely on crop varieties that deliver greater sustenance, productivity and/or profitability, and are environmentally stable to reduce risk of potential crop loss in their adoption. Commercial and public breeding efforts have been successful over the past 100 or more years in improving the yields and often the quality of our major food and fibre crops [1]. This concerted breeding effort follows in many crops from access to germplasm representing early human-guided selection during domestication [2]. Over many years, selection from the original wild progenitors has affected changes in gene frequency for traits including seed dormancy, seed retention/shattering and threshability, and agronomic performance including lodging, grain yield and its components grain size and number [e.g. [3–5]. Such crop improvements have often narrowed the genetic base within many breeding programs and there is evidence that diverse genetic resources (GR), including landraces, wild relatives and synthetics, have the potential to increase population variance for traits important for continued crop improvement. Recent advances in high-throughput field phenotyping (HTFP) has enabled the capacity to non-destructively and remotely-sense crop traits in a high-throughput fashion to accurately characterise populations containing many thousands of individuals [6,7]. Herein we report on the capacity to deploy HTFP to enrich populations developed from GR for genetically-complex traits in crop improvement programs. The paper highlights how breeding lines developed from GR and once discarded owing to poor adaptation or other unfavourable genetic linkages can be identified, retained and recycled if targeting novel alleles contributing to traits underpinning crop performance. Many of the examples cited herein are for wheat but the opportunities for improved and reliable trait assessment in GR can be

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readily extended to most crops.

1.1. The issue with constrained genetic diversity

In both self- and cross-pollinated crop species, breeding has maintained genetic gain through the selection and substitution of 'poor' for 'good' alleles, and specific allelic (epistatic) combinations [8]. The commercial varieties grown today can be thought of simply as reflecting pockets of intensive cycling and enrichment of adaptation alleles through parental identification, hybridisation, and population development. The focus within individual breeding programs is the building of gene pools enriched for key alleles underpinning local or regional adaptation (including climate and subsoil constraints), disease and insect resistance, and grain/seed quality [8]. While these activities are separated geographically and genealogically across the globe there are sets of key genes under continued selection and are often ubiquitous especially when breeding for intensive farming systems including dwarfing and development/synchrony genes, shoot and root diseases [5]. While much of this germplasm is shared through collaboration and via the international research centres (e.g. the Consultative Group for International Agricultural Research (CGIAR) centers), intensive breeding and improved selection methods risks constraining genetic diversity [e.g. [9,10].

History has shown the dangers of reduced genetic diversity and the risks of genetic vulnerability (e.g. maize T-cytoplasm linked southern corn leaf blight hypersensitivity; [11]). Similarly, reduced additive genetic variance will slow gain from selection to reduce genetic improvement. The promise of genomic selection and the capacity to hasten the period between consecutive breeding cycles while directly selecting for genomic regions underpinning target traits, has potential to rapidly reduce genetic variation in already elite, albeit restricted, breeding populations. Thus, continued genetic gain is becoming increasingly reliant on access to increased genetic diversity and new alleles through diverse GR together with gene technologies including mutation, tilling, genetic modification (GM) and new CRISP/Cas9 methods [12,13]. The use of GR is widely cited [e.g. [14,15] yet while much has been said of the potential for GR [e.g. [16,17], their adoption in commercial breeding has been limited [18] with few examples beyond new major genes for disease and insect pests [19].

1.2. The utility of diverse genetic resources

Domestication represents human-induced change with selection from originally wild plant populations. Resulting changes in gene frequency underpin the genetic make-up of crop varieties grown today, and commencing with the identification and retention of select plants together representing a small portion of the potential genetic diversity currently available in crop genetic resource gene banks [20–22]. The potential here is realised when it is considered that greater genetic variability was observed in a single self-incompatible, wild tomato relative than collectively all self-compatible, wild tomato accessions [23]. Further, marker-based studies indicate cultivated tomato (*Lycopersicon esculentum* L.) contains less than 5% of the genetic variation contained in its wild relatives [24].

Agricultural fitness is very different to natural fitness contrasting elements of productivity and survival, and the promotion from natural ecotypes to landraces reflecting a focus on genetics (and primarily major genes) underpinning harvestability (greater seed retention, modified plant/crop architecture, seed size and threshability – [25]). Increased reproductive fitness will have promoted selection of genes and changes in gene frequencies reflecting greater seed numbers, yet despite these the focus on managed agriculture and factors promoting the widespread migration of crops will likely have promoted genic sampling through specific founders and potential for genetic bottlenecks in many of our modern crops [20].

The various gene banks, core and other GR collections (e.g. the Vavilov and Watkin's collections in wheat [26]) are likely to contain many traits and the underlying genes not yet sampled and unique of those contained in commercial crop breeding programs [27]. Similarly, the use of geographic information to identify wild or landrace-based germplasm with uniquely local adaptations (Focused Identification of Germplasm Strategy or 'FIGS') has extended the relevance of targeted genetic diversity and capacity for allele mining available to crop breeders [28]. Supporting this opportunity are modern genetic techniques allowing the transfer of genes across increasingly wider genetic barriers to hybridisation characteristic of the secondary and tertiary gene pools. For example, global deployment and use of novel genes for stem rust resistance and improved grain yield contained in the 1B.1R wheat-rye translocation was compromised by undesirable dough quality associated with the rye secalin gluten proteins. Efforts to break linkage between stem rust resistance and secalin genes using various genetic strategies were reviewed [29], and hybridization barriers between wheat and rye overcome and chromosomal linkage broken through application and analysis of molecular markers [30].

Ongoing genetic progress relies on the assessment, identification and incorporation of new traits and trait combinations from both elite and GR, with targeted deployment into the breeding gene pools. Many invaluable major genes have been identified and used in the development of improved crop varieties, and wheat (*Triticum aestivum* L.) especially has been a successful recipient of important alleles necessary to maintain improved adaptation, pest and disease resistance (Table 1). A notable example are the *Rht1* (syn. *Rht-B1b*) and *Rht2* (syn. *Rht-D1b*) green revolution, wheat dwarfing genes first introduced into Japanese wheat varieties from the Korean landrace Daruma. These genes were collected after World War II and introduced through the Japanese dwarf variety Norin 10 into Dr Orville Wright's USDA Washington State Wheat breeding program in 1949 before their deployment globally through Dr Norman Borlaug and the CIMMYT wheat breeding program. Since their initial distribution and uptake, other gibberellin-responsive dwarfing genes have been identified and used (e.g. *Rht18*) from GR developed in wide-scale, mutagenesis-based breeding efforts throughout the world [31].

Yet despite the significant contribution from major genes, the potential for GR for multiple small effect alleles underlying genetically complex traits has been of limited success in release of commercial crop varieties [18]. Some notable exceptions include Californian tomato varieties containing small chromosomal segments of *Lycopersicon pennellii* and Chinese 'super-rice' hybrids containing *Oryza rufipogon* chromosomal segments [1,50]. An *Avena sterilis* cytoplasm was used in the release of a high-yielding, commercial oat variety Hamilton [51]. The major reason for the poor uptake of quantitative traits in the GR is that genetically diverse populations representing the GR commonly contain germplasm representing landraces and wild relatives poorly adapted to mechanised agriculture. Their excessively tall height, very late flowering (and maturity), lodging and disease susceptibility make phenotyping and assessment of alleles challenging in commercial breeding programs focussed on highly-adapted genotypes (e.g. Vavilov collection in Plate 1). Further, chromosomal linkage contributing to linkage drag and negative physiological associations limit the utilisation of GR and slow the timeframes in delivering elite breeding lines derived from diverse genetic sources [18]. Ideally, evaluation and selection should commence following one and sometimes two rounds of backcrossing to an elite commercial variety [18,49,52] and as shown for GR- (landrace and wild relative) derived backcross lines in Plate 2. Availability of molecular markers supporting background selection and/or progression of multiple BC-derived F1s should assist in maintaining allelic diversity from the GR donor(s) while selecting alleles and recovery of adaptation in the recipient genetic background [53].

1.3. High-throughput field phenomics (HTFP)

High-throughput field phenomics (HTFP) represents the capacity to

Table 1
Traits and sources of novel genetic diversity currently in use in commercial wheat breeding programs.

Trait	Gene/phenotype	Diversity source/donor species	Reference
Grain quality	*GPC-B1 (grain protein)*	Landraces (*T. dicoccoides* Korn.)	[32]
	Grain milling quality	Synthetics (*Ae. tauschii* Coss.)	[33]
Soil chemical constraints	*Nax 1, Nax 2 (salt tolerance)*	Landraces (*T. monococcum* L.)	[34]
	Increased nutrient uptake	Landraces (*T. aestivum* L.)	[35]
Leaf diseases	*Lr32 (leaf rust)*	Wild relatives (various)	[36]
	Sr2 (stem rust)	Wild relatives (*T. dicoccoides* Korn.)	[37]
	BYDV (virus)	Wild relatives (*Thinopyrum Intermedium* Host Barkworth & Dewey)	[38]
Head diseases	Fusarium head blight	Landraces and wild relatives (various)	[39]
Insect pests	Hessian fly	Wild relatives (*Ae. tauschii* Coss.)	[40]
	Russian wheat aphid	Wild relatives (*T. dicoccoides* Korn.)	[28,41]
Weed competitiveness	Greater shoot/root vigour	Landraces (*T. aestivum* L.)	[42,43]
Biological nitrification inhibition	Reduced soil nitrification	Wild relatives (*Leymus racemosus* Lam.)	[44]
Heat tolerance	Various	Synthetics (*Ae. tauschii* Coss.)	[45]
		Landraces (*T. aestivum* L.)	[46]
Drought tolerance/WUE	Various	Rye (*Secale cereale* L.)	[47]
		Synthetics (*Ae. tauschii* Coss.)	[48]
		Landraces (*T. aestivum* L.)	[48]
Grain yield potential	Various	Synthetics (*Ae. tauschii* Coss.)	[49]
	Rht-B1b, Rht-D1b (dwarf genes)	Landraces (*T. aestivum* L.)	[30]
	Rht13, Rht18 (dwarf genes)	Mutant donors (*T. aestivum* L.)	[31]

'WUE' = Water-use efficiency.

Plate 1. Nursery contrasting phenotypic diversity in a modern commercial wheat (foreground) with diversity contained in a 'spring' enriched component of the Vavilov collection (background).

Plate 2. Phenotypic diversity across backcross lines (here BC1- and BC2-derived) developed from crosses between Australian commercial wheat parents and a range of high biomass landraces.

non-destructively and remotely-sense crop traits in a high-throughput fashion to accurately characterise populations containing many thousands of plants and plots [6,7]. In contrast to traditional phenotyping-by-eye, the mainstay of selection in breeding programs over the past 100 or more years, HTFP aims to partially automate and thereby reliably quantify previously routine measures of a plant or plot's phenotype and underlying genotype in response to environment and/or agronomic practise. The value-add with HTFP in crop breeding is the capacity to extend from hundreds to potentially thousands of genotypes within and across multiple environments, and across multiple timescales [7]. Recent advances in data analytics and computational capacity may then improve population-based prediction models in developing phenotype-based selection indexes and with pedigree information estimates of breeding value or genomic models, including genomic selection when integrated with population genetic data [8]. However, while much has been promised in the potential for HTFP in breeding [e.g. see [7,54,55], publications to date have focussed on genetically-fixed varieties evaluated in large-sized plots. Few reports have addressed the capacity for HTFP in improving efficiency in selection in large, segregating breeding populations relevant to the challenges in commercial breeding where, in early generations the availability of only a small amount of seed per line often limits plot size to hill-plots or a couple of short rows [56]. Further, while the application of HTFP in large plots appears relatively straightforward, such application does not demonstrate the opportunity and potential particularly when breeders routinely harvest large plots for yield as a target in itself.

Phenomics broadly includes phenotyping capacity in both controlled environments (CE) [54] and the field environment [7]. Controlled environments (CE) have been used successfully to reliably phenotype novel yet difficult-to-measure or manage plant traits present within the GR (e.g. salt exclusion and tolerance in tetraploid wheat landraces ;[34]). However, there is a strong level of scepticism in the use of CE in targeting genetically complex traits especially in advanced developmental stages where response in a spaced pot is uncorrelated with performance in a plot where plants compete with neighbours (see review by [56]). In turn, the focus of this paper is to highlight the capacity in HTFP to reliably characterise those phenotypes underpinning both genetically simple and complex traits under assessment in field conditions. Managed field environments [57] aid in developing and characterising environments in a way that the phenotypes are relevant to a specific challenge (e.g. drought, nutrient deficiency or temperature

extreme), or more broadly representative of the total target population of environments.

Detailed high-throughput plant phenotyping and characterisation was first highlighted for CE [54] but has now has been developed for wide-scale use in the field where complex traits such as leaf area, biomass and potentially grain yield can be phenotyped cheaply and reliably on potentially many hundreds to even thousands of plots [58]. By reliably parameterising phenotypic variation among genotypes in the field, confidence is increased in the genetic correlation of trait performance to other field environments including breeder's nurseries and advanced breeder's trials. HTFP has potential to non-destructively and remotely-sense from single leaves and ground cover display [59], to canopy architecture and leaf senescence [58], crop biomass [58] and aerial-based, thermal-imaging as a surrogate for crop transpiration [61,62]. Other key targets have been identified including plant height and indirect estimates of biomass including NDVI [63] yet the value and reliability of these as useful to crop breeding and/or in predicting key traits can be sometimes questionable [58,64,65]. provides an excellent summary of the different remote-sensing technologies available for HTFP in breeding programs. Further while there is capacity for many physiological, morphological and botanical attributes to be phenotypes with phenomics, their use and value must be assured. The concern in contrasting what *can* be done as 'recreational phenotyping' (sometimes described as 'stamp collecting') and what is of value in research as 'targeted phenotyping' must be demonstrated around a clear value proposition. Phenomics must have capacity to deliver especially in crop breeding where costs in large-scale phenotyping can be considerable (see later).

1.4. Deploying high-throughput field phenomics in identifying useful GR derivatives

Breeders aim to increase genetic gain through any method available to them. The capacity to maintain genetic gain reflects a masterful ability to identify, take-up and efficiently integrate across a range of genetic, statistical and phenotyping tools that lead to: (1) the identification and development of large populations containing new and relevant genetic diversity while maintaining a high population mean; (2) the reliable and confident characterisation of germplasm contained in these populations; and (3) the capacity to identify elite lines in a truncated period of time or 'cycle'. Together these activities can be summarised mathematically as the 'breeder's equation':

$$\Delta G_y = h_y^2 \times s_y \times k$$

where ΔG_y is the genetic gain relative to the population mean from direct selection for trait y, h_y^2 is the narrow-sense heritability and s_y the phenotypic standard deviation of trait y, and k the selection differential (for a normally-distributed population) in standard deviation units. By including t as the generation time between successive cycles of selection, genetic gain per cycle for direct selection becomes:

$$\Delta G_y = (h_y^2 \times s_y \times k) \times t^{-1}$$

In influencing any component of the genetic gain formulae, breeders can increase genetic variance through genome editing, mutagenesis, or the introduction of novel genetic variation including GR. While gene editing promises so much it, like mutagenesis, is contingent on identification of targeted single gene events [66]. By contrast, crop wild relatives and landraces contain potentially large and uniquely new genetic diversity for qualitative and quantitatively-inherited traits with potential for use in breeding (e.g [27,51]).

Almost all elements of the genetic gain formulae rely directly or indirectly on phenotyping. Phenotyping represents the characterisation of part of or the entire plant or crop, and as such is commonly slow, costly and variable in quality and reliability and thereby sometimes contributing to reductions in heritability and the correlation of what is observed and the underlying genotype - the basis for selection and genetic gain. HTFP assists breeding in: (1) increasing heritability with multiple measurements (to reduce the sampling variance); (2) better characterising of environments to manage genotype × environment interaction and their correlation in the target population of environments; (3) reducing cycle time with earlier testing in spaced-plants or single rows; and (4) in the capacity to increase phenotypic variance through extending from assessment of hundreds to potentially thousands of GR and GR-derived genotypes within and across multiple environments, and across multiple time-scales [58,65].

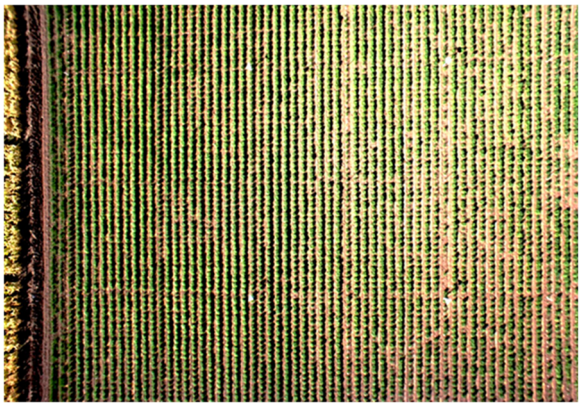

Plate 3. Aerial view of spaced hill-plots each separately containing a wheat GR germplasm growing and competing side-by-side at Obregon Mexico (Source: Dr Matthew Reynolds, CIMMYT El Batan Mexico).

The application of HTFP in large plots while straight-forward does not demonstrate the opportunity and potential for Phenomics particularly when breeders routinely harvest large plots for yield as a target in itself. The robustness and capacity for repeated measures with HTFP permits confidence in phenotyping earlier stages of breeding when seed per line is limited, plots are small, heritability is lowest, and the bottleneck in genetic variance through restricted population size is greatest. By allowing reliable direct or indirect selection in early generations breeders can increase the size of their breeding nurseries thereby allowing for greater genetic diversity (see Plate 3). Increasing selection intensities will allow culling for the more expensive replicated testing in later stages of commercial testing while maintaining key and possibly rare alleles from novel genetic resources for recycling back into the breeding program for use in population development and later progeny assessment.

Table 2 summarises potentially useful variation for complex traits contained within the GR, the remote-sensing and other technologies available for their HTFP, and the capacity to scale reliably to smaller units when phenotyping. The value in HTFP in phenotyping larger, multi-row plots is well established (see [65]), whereas scaling to smaller units such as single plants, spaced-hills and or single/paired rows is less well established yet potentially where the greatest benefit in breeding is likely to occur. This is especially true of GR and their derivatives where seed numbers are commonly few because of poor adaptation or genetic issues with fertility [2,18]. For many technologies extension to smaller plots is straightforward requiring a minor change in data capture and analytics.

A key concern in phenotyping smaller plots is in the potential for intergenotypic competition as the GR are commonly very diverse in vigour, canopy size and development (Plates 1–3Plate 1). This competition can make the phenotyping of genetically-complex traits and particularly those associated with canopy development, biomass and grain yield challenging as the environment of one genotype can be influenced by a neighbouring and competing genotype. Competition for water, light and nutrients necessary for canopy growth will be variable where immediate neighbours are genetically different, and particularly under stress conditions where competition for resources and influence

Table 2
Potentially useful variation for traits contained in diverse Genetic Resources (here cereals) and the plot scale required to reliably phenotype using high-throughput technologies.

Trait/phenotype	Phenomics tool	Single/paired row	Multi-row plot
Establishment/ground cover	RGB, LiDAR, NDVI	Yes	Yes
Canopy development/senescence	LiDAR, NDVI, RGB	Yes	Yes
Anthesis biomass	LiDAR	Yes	Yes
Harvest index	LiDAR, hyperspectral imaging	Possibly	Possibly
Photosynthesis/transpiration	Thermal-imaging, fluorescence	Yes	Yes
Nutrient uptake/use-efficiency	Hyperspectral imaging	Possibly	Yes
Ear/spike number and growth	LiDAR, RGB	No	Yes
Canopy lodging	LiDAR/RGB	No	Yes
Plant/canopy height	LiDAR	Yes	Yes
Canopy stay-green	LiDAR, NDVI, RGB	Possibly	Yes
Pests and disease incidence	LiDAR, RGB	Yes	Yes
Weed competitiveness	LiDAR, RGB, hyperspectral imaging	Possibly	Yes
Drought tolerance	Thermal imaging, LiDAR	No	Yes
Temperature extremes	LiDAR, thermal imaging	Yes	Yes

LiDAR = Light Detection and Ranging; NDVI = Normalized Difference Vegetation Index; RGB = Red Green Blue imaging.

on productivity greatest [56,67]. That aside, small plots including hill-plots and single-rows have been used in selecting in early generations for grain yield in oats [68] and in wheat [69,70]. However, care must be taken in the genetic correlation of the selection and target environments, and the opportunity in use of common borders to reduce interplot competition [56].

1.5. Indirect selection

In a breeder's nursery yield is almost always the primary target and is weighted heavily in any index or ranking of breeding lines when considering promotion to the next stages of testing prior to varietal release. While most efforts are considered toward directed selection for traits such as yield, indirect selection for target traits via component physiological, morphological or biochemical traits can provide many benefits to breeding programs, such as the opportunity to introduce new alleles to maintain genetic variability from which genetic progress can be made, and importantly, an inexpensive means for early generation selection of one or more important drivers of adaptation (e.g [56,71].). The benefits of trait-based selection have been demonstrated in enriching populations for performance in water-limited environments (e.g [72]), and more recently in complementing marker-based drivers of performance in genomic selection [62,73].

The genetic gain for indirect selection for a target trait y per cycle via an associated trait i, $\Delta G_{i,y}$, selected in the same population is a simple extension of the breeder's equation and is given mathematically as:

$$\Delta G_{i,y} = (h_i \times h_y \times r_a \times s_y \times k) \times t^{-1}$$

where the terms are as before except h_i and h_y are the square roots of the narrow-sense heritabilities of the selected trait i and the responding or target trait y, r_a is the additive genetic correlation between the selection and target traits, and s_y the standard deviation of the target trait. The opportunities with indirect selection in breeding with GR is reviewed extensively in [56].

Two examples are now provided highlighting the capacity for HTFP in characterising populations of GR-derived breeding lines for traditionally difficult-to-phenotype traits important in improving crop yield.

1.6. Total biomass

Crop breeding programs globally have been remarkable in maintaining linear increases in grain yield across a range of crops [1]. In the inter cereals, ongoing yield increases has largely reflected selection for greater partitioning (or 'harvest index'; HI) to affect increases in grain number (e.g [74].). However, in many regions, HI is reaching toward a theoretical maximum [75] reducing the ongoing capacity for genetic gain in this yield component. Grain yield represents the partitioning of total biomass to grain yield yet efforts to change total biomass in selection for greater yield have been negligible in some breeding programs (e.g [74].) with some reports suggesting an increase in biomass in others [e.g. [76,77].

Crop biomass represents the totality of biological growth, and the net integration across four distinct physiological components: (1) time to full ground cover; (2) radiation-use efficiency during full ground cover; (3) duration of full ground cover; and (4) maintenance of canopy stay-green through grain-filling. Biomass then represents growth across the entire season with small efforts to modify growth potentially realising capacity to increase grain yields without changing partitioning through harvest index. Biomass itself is difficult to measure as sampling requires large plots and so selection is delayed to later stages of selection where seed is plentiful and replication can be undertaken. As early growth and biomass are under strong additive genetic control [78], selection for greater biomass can be undertaken at early stages of inbreeding as long as seed is plentiful and phenotyping reliable. However, heritability for biomass is low even on large plots owing to the subsampling and drying of a smaller representative area of the plot [72]. HTFP allows improved assessment of biomass in full plots as the entire plot area and not a 'representative area' is predicted for biomass while there is capacity to undertake multiple passes to reduce the sampling variance and increase confidence on biomass estimation. Importantly, HTFP also permits reliable assessment and screening much earlier in the breeding cycle when genotypes are grown in smaller plots or rows (Table 2).

The GR have previously been identified as an excellent source of increased biomass. In wheat, landraces have been demonstrated to contain greater biomass than commercial wheats in Iran [79] and in Mexico [80]. Interspecific synthetics derived from wild goat grass (*Aegilops squarrosa*) also produced significantly greater biomass than commercial checks contained in the same study [80]. In Iowa, Dr Ken Frey used wild oat relatives (*Avena sterilis* L.) to increase genetic diversity in spring oats for biomass and grain yield across multiple recurrent selection populations [51]. Here large numbers of families were screened in early generations in hill plots and then validated in larger plots to increase grain yield by up to 30% while maintaining appropriate phenology and HI. Similarly, in backcross-derived populations developed from wild and other diverse sorghum germplasm [81], increased biomass to significantly increase grain yield above recurrent parents while maintaining appropriate height and phenology. In backcrossing from unadapted genetic sources, reasonable genetic

variation was maintained to increase genetic diversity for many traits in the sorghum breeding program.

Low cost, HTFP technologies such as LiDAR (Light Detection and Ranging) permit the reliable and rapid yet non-destructive assessment of biomass, canopy height and green leaf area [e.g. [58,60,82]. At CSIRO in Australia, we have mounted LiDAR units on a self-propelled, lightweight and portable frame called the 'Phenomobile Lite' [58] (Plate 3). This unit permits indirect assessment of biomass, green leaf area and numbers of spikes of up to 700 plots per hour (assuming an average 5 s per 6 m-long plot). Data are uploaded and a data analytics pipeline returns 3D-point cloud data from which developed prediction models provide growth estimates.

We grew 55 random landrace and commercial wheats in unbordered rows under disease-free, rainfed conditions in the field. A single LiDAR run was undertaken using the Phenomobile Lite platform (Plate 3) on 36cm-spaced, 1m-long rows in an augmented experimental design at anthesis. The row was then cut and numbers of spikes counted before drying at 72 °C for four days in a fan-forced oven before weighing. The LiDAR 3D-point cloud data were converted to a predicted biomass after [58], and row-cut dry matter and numbers of spikes expressed on a unit area (m^{-2}) basis (Fig. 1). The GPS positioning system contained on the Phenomobile Lite permits easy identification of the row and the subsequent prediction of row biomass, plant height, and green leaf area and its distribution.

The range among lines in total biomass and numbers of spikes was three- and five-fold, respectively, reflecting the large phenotypic variation commonly observed for growth-based parameters in GR (Plate 1). Relations were strong and linear for single-row, LiDAR-predicted biomass and total biomass ($r = 0.82$, $P < 0.01$) and numbers of spikes ($r = 0.66$, $P < 0.01$) (data not shown). Total biomass and numbers of spikes were themselves strongly and positively correlated ($r = 0.83$, $P < 0.01$) as were measured and predicted plant height ($r = 0.98$, $P < 0.01$) (data not shown).

The LiDAR-based prediction equation for biomass was developed for large plot canopies representing a range of commercial and advanced wheat breeding lines [58]. In Fig. 1, LiDAR did predict effectively ($r^2 = 0.44$ to 0.66, $P < 0.01$) across varying genotypes and plot scale for the two complex biomass and numbers of spikes phenotypes. Further, the relationships were strong given the varying canopy heights and canopy architectures representing the diverse sources of germplasm represented (data not shown). The value in such predictions would allow for non-destructive measures of biomass including temporal changes in biomass accumulation to quantify dynamic values of RUE and final biomass at low cost. Such values are incredibly challenging for large numbers of lines and especially difficult where agronomic type

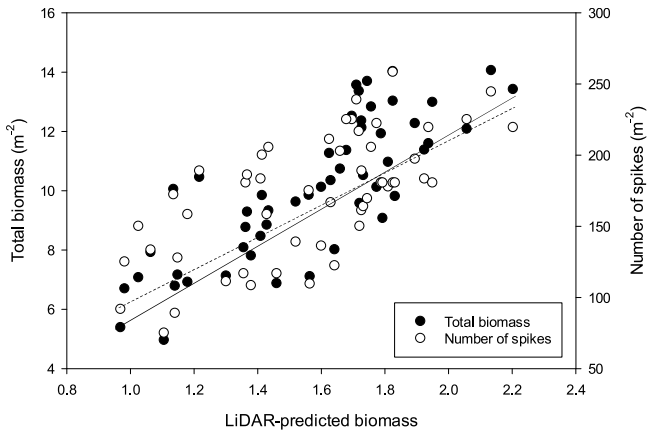

Fig. 1. Anthesis relationships of single-row, LiDAR-predicted biomass (t/ha) and (1) measured biomass (•, —, $Y = 0.10 + 6.35\ X$; $r^2 = 0.67$, $P < 0.01$), and (2) spike count (o, —, $Y = 26 + 93\ X$; $r^2 = 0.44$, $P < 0.01$) for 55 spring landraces and commercial varieties grown under favourable conditions in the leading up to and after flowering makes phenotyping challenging. This is commonly the case with lodging and disease, and leaf-firing/senescence common to unadapted germplasm and including responses not uncommon in the GR and their derivatives [18,83].

1.7. Canopy temperature

Increased photosynthesis and greater water-use efficiency are targets in crop breeding and physiological efforts to increase crop biomass and grain yield [84]. Wild relatives and their derivatives have been reported to contain traits linked to greater canopy photosynthesis [e.g [49,85]. and may have potential in delivering new nuclear and cytoplasmic alleles, and their cytonuclear interactions [e.g [86]. for improved photosynthesis to breeding programs. Yet despite their value both photosynthesis and stomatal conductance are slow and difficult to measure, and so cost-effective surrogates for each are desirable in screening breeding populations. Transpiration is closely related to photosynthesis through stomatal conductance [87] while genotypic variation in transpiration reflects water access and water-use, and the capacity to affect transpiration efficiency and grain yield in water-limited environments [88,89].

Canopy temperature (CT) and canopy temperature depression (CTD) are used to reliably measure leaf or canopy transpiration as a surrogate for leaf conductance [91,92] and grain yield [85]. Previously, hand-held infra-red thermometers permitted sampling of a crop canopy in 7–10 seconds. Together with movement between experimental plots, a modest-sized population of 300 entries replicated twice would require a skilled operator *c.* 120–150 minutes in order to sample. The sensitivity of stomata to small microclimate changes in wind speed, VPD, and cloud cover etc. (see Fig. 2) will introduce significant environmental variation to reduce heritability and confidence in genetic differences when sampling over a protracted period [92]. Hand-held thermal sensors commonly produce heritability estimates for CT of less than 10% [92] substantially reducing the correlation in phenotypic and underlying genotypic values. Airframe-mounted infrared cameras permit with thermal resolution to approximately $10cm^2$ while allowing potentially many 100 s and even 1000s of plots to be surveyed for CT in the space of a few seconds (Plate 4). The shorter timeframe over which an experiment or nursery is sampled for CT removes confounding through subtle changes in microclimate [61]. In turn, plot-based heritabilities obtained from helicopters flying at an altitude of 100 m are in the order of 60–80% increasing confidence in detecting among plot and genotype differences [61,62].

Plate 5 summarises diversity in CT for a study containing overseas wheat introductions and modern wheat varieties grown in large $10m^2$ plots in the field. The image was taken immediately after anthesis in an irrigated experiment with a single aerial pass early in the afternoon (around 1 pm) using a helicopter-mounted, portable Helipod platform

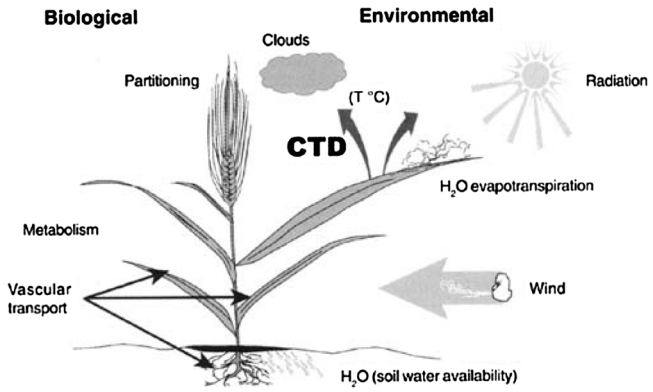

Fig. 2. The challenge in accounting for the many factors affecting canopy temperature depression (CTD) in plants (copyright [90]).

Plate 4. A self-propelled, portable Phenomobile Lite ® containing a LiDAR, RBG camera, Greenseeker ® and GPS-tracking in operation in the Yanco NSW Managed Environment Facility.

Plate 5. A heliframe carrying a pod containing GPS and high precision FLIR SC645 thermal infrared camera.

containing a high precision, FLIR SC645 thermal infrared camera [61]. The range in plot temperatures was 2.9 °C with plot temperatures themselves contrasting strongly with the much warmer soil temperatures between plots and on the laneways adjacent to the experiment (+ 15 °C, shown as an orange hue). Crop lodging common to the taller DGR is seen in the merging of colour hues across some adjacent plots while small lighter hued squares in each plot indicate regrowth of plants after biomass sampling earlier in the season (Plate 6).

Heritability for this flight was 73% (data not shown). Heritabilities for aerial thermo-imaging are routinely high [61,62] compared with low heritability estimates reported for CT and CTD obtained using hand-held infrared thermometers [e.g. [88,92]. The higher heritabilities indicate greater confidence in among-genotype and -family comparisons for CT and stomatal conductance, and greater confidence in: (1) selecting among families for altered CT, and (2) genetic/physiological architecture and factors underpinning biomass, yield and water-productivity in targeted populations [e.g. [87,93]. Further, the short time-frames and reduced cost of CT per plot with aerial thermo-imaging permits regular measures throughout the day and across days to understand the impact of changing soil water potential, aerial temperature, VPD and hydraulic conductance on CT and stomatal conductance across genotypes [61].

While the operating cost of thermal infrared cameras mounted on a helicopter may be too expensive for some breeding programs it is

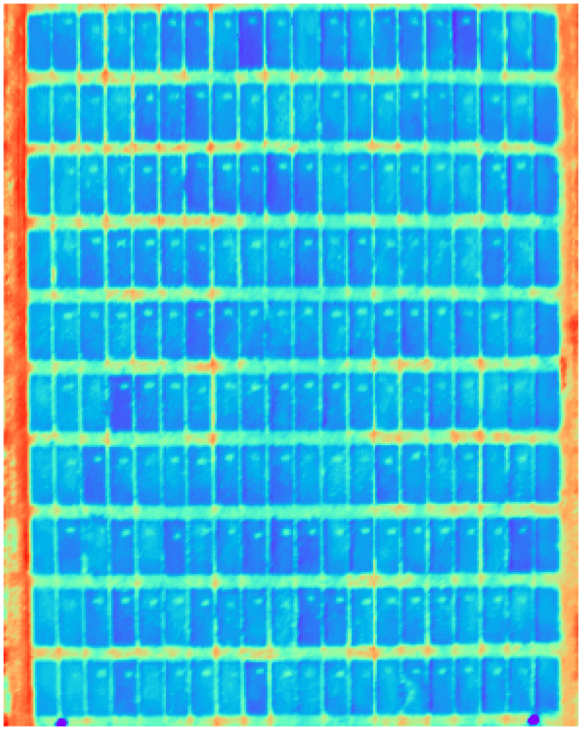

Plate 6. Visualisation of canopy temperatures (CT) collected on a diverse set of overseas GR introductions and modern wheats using a high precision FLIR SC645 thermal infrared camera mounted on a helicopter travelling in a single pass above the experiment. The range in CT for genotypes across the experiment was *c.* 3 °C with darker hues associated with cooler canopies. Small, lighter-blue squares are CT associated with crop regrowth following earlier sampling.

notable that the cost per plot, when estimated on a modest 3000 plots of size 10m^2, equates to *c.* US$0.30 per plot (please refer [61]). Given the potential with ongoing technology improvements to further reduce the cost per plot together with the appreciably high heritabilities obtained, airborne CT and derived CTD could be a cost-effective HTFP method for use within breeding programs. Other options to helicopter-mounted thermal-imaging cameras in smaller breeding programs include the use of unmanned aerial vehicles. The benefits here are in further cost reduction and/or in remote locations where manned helicopters may be unavailable. However, the main limitation here is in the radiometric quality of the images obtained from smaller sensors where thermal drift remains a significant concern [94]. Here substantial effort is still required to ensure adequate accuracy in robust temperature estimates not impacting on reductions in heritability.

1.8. Data handling

The significant scale required to adequately assess CGR and their derived progeny is highlighted in Plate 3 where replicated experiments undertaken across multiple environments will produce large volumes of field data for analysis. Phenomics compounds the issue of data volume and the challenge of data handling in successful deployment of many HTFP systems. The successful deployment of HTFP within publically-funded research programs has relied on a multi-disciplinary collaboration including capabilities such as remote sensing, software engineering and statistics (e.g. see [58,61,62,95],). In these examples dedicated data processing pipelines were developed and used to translate and segment large volumes of raw data (e.g. from images and scanners) into phenotypes for each field plot. The ongoing development of dedicated data handling systems for HTFP together with the increased availability of data handling and analysis scripting software

(e.g. Python (www.python.org) and R (www.r-project.org)) for scientists should alleviate the data handling challenges and increase availability of HTFP to breeding programs.

2. Conclusions

The GR have demonstrated capacity to deliver new alleles for major genes yet the potential exists for new as yet untapped genetic diversity underpinning traditionally difficult-to-measure traits such as leaf area and its distribution, biomass and stomatal conductance. Thoughtful population development and selection in early generations will permit reliable evaluation in smaller plots when seed is sparse using HTFP for the drivers of yield, and especially those novel and rare alleles commonly lost when targeting grain yield alone in breeder's nurseries. The identification of novel phenotypes and the retention of those unique alleles will enable their being recycled through subsequent crossing and population development. Further, the targeting of novel phenotypes using rapid, cost-efficient HTFP will also allow for larger nurseries containing greater numbers of GR-derived lines and their assessment independent of grain yield. Together, integration of HTFP with deployment of GR will increase genetic variance and improve precision while reducing the cost of phenotyping, and hastening the period between breeding cycles to better utilise this important genetic resource.

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